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The plaque-inhibiting capacity of 11 antibacterial compounds

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The plaque inhibiting effect *in vivo* of 11 antibacterial agents was compared with their antibacterial activity against salivary bacteria *in vitro*. The *in vivo* effect was tested for 4 days in a human model with a supplement of sucrose in the diet. The antibacterial activity of the compounds, which were chosen from different main groups of disinfectants (alcohols, iodophores, dyes, quaternary ammonium bases, amidines, guanidines) were tested in four *in vitro* systems. No correlation was evident between the *in vivo* and *in vitro* effects. Chlorhexidine gluconate and -acetate proved most effective *in vivo*, whereas several other substances equally or more effective against salivary bacteria *in vitro*, exhibited no effect *in vivo*. It is concluded that other factors than the antibacterial properties are important in plaque inhibition *in vivo*.

Introduction

Bacterial plaque on the teeth is generally regarded as a dominant etiological factor in caries and periodontitis: Gingivitis develops within 2–3 weeks if plaque is allowed to accumulate at the gingival margin (Löe, Theilade and Jensen 1965), and after a period of about 4 weeks without oral hygiene, early carious lesions may be detected on tooth surfaces covered with plaque (v.d. Fehr, Löe and Theilade 1970).

Mouthrinses with various antibacterial agents have been shown to reduce the number of bacteria in the saliva temporarily (Slanetz and Brown 1949, Strålfors 1961). In hamsters and rats some disinfectants have effected a reduced incidence of caries and periodontitis when added to the drinking water or applied topically (Keyes et al. 1966, Strålfors 1967 a, b, Regolati, König and Mühlemann 1969). Renggli (1966) and Schroeder (1969) reported an inhibiting effect on formation of dental deposits in man by mouthrinses with chlorhexidine acetate, whereas a total inhibition of plaque was observed by Löe and Rindom Schiøtt (1969, 1970a,b) using 0.2 per cent chlorhexidine gluconate.

In previous studies very few of the antibacterial substances tested have been found to be effective plaque inhibitors *in vivo*. In the present experiment a comparison has been made between the plaque inhibiting effect of 11 compounds *in vivo* and their activity against salivary bacteria *in vitro*. The substances were chosen from various main groups of antimicrobial agents (alcohols, iodophores, dyes, quaternary ammonium bases, amidines, quanidines) to evaluate the effect of different antimicrobial

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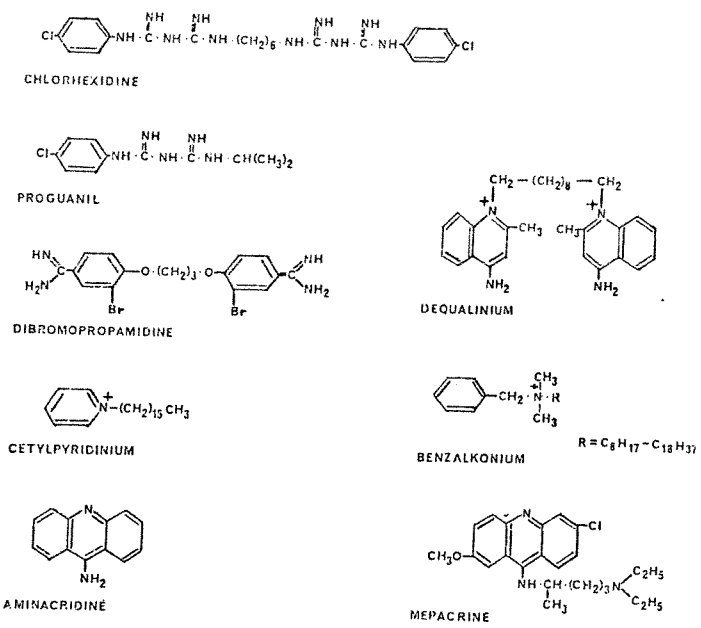

Fig. 1. The formulas of some of the antimicrobial compounds tested.

principles in the mouth. The majority of the compounds tested were cation active.

Material and Methods

The following antimicrobial compounds were tested: *chlorhexidine digluconate** and *-diacetate* [1,1′-hexamethylenebis (5-(p-chlorophenyl) biguanide) digluconate and -diacetate], *Jodosan,®* *cetylpyridinium chloride*** [1-hexadecylpyridinium chloride], *ethanol, dequalinium chloride* [decamethylenebis (4-aminoquinaldinium) chloride], *benzalkonium* chloride, *aminacridine hydrochloride* [9-aminoacridine hydrochloride], *mepacrine hydrochloride* [3-chloro-7-methoxy-9-(1-methyl-4-diethylaminobutylamino) acridine dihydrochloride], *hydrogen peroxide, proguanil hydrochloride* [1-(p-chlorophenyl)-5-isopropylbiguanide hydrochloride] and *dibromopropamidine diisethionate* (Fig. 1). [2,2′-Dibromo-4,4′-diamidinodiphenoxypropane di-(β-hydroxyethanesulfonate)].

Clinical tests. Sixty dental students, average age 19 years, volunteered for the experiment. All the students were, after having had a thorough prophylaxis, instructed to suspend oral hygiene for 3 days. To provoke plaque formation, sucrose rinses were instituted (Carlsson and Egelberg 1965, Carlsson and Sundström 1968). The students rinsed with 10 ml. 15 per cent w/v sucrose for 1 minute every second hour between 8 a.m. and 10 p.m. At the end of this period the amount of plaque accumulated on the teeth was estimated by use of the Plaque Index (Pl I) (Silness and Löe 1964). The 16 individuals showing the highest Pl I values were selected to form a shorttime model for studying plaque formation *in vivo* (Fig. 2).

* ICI, Macclesfield, England
** Pyrisept®, Weifa.

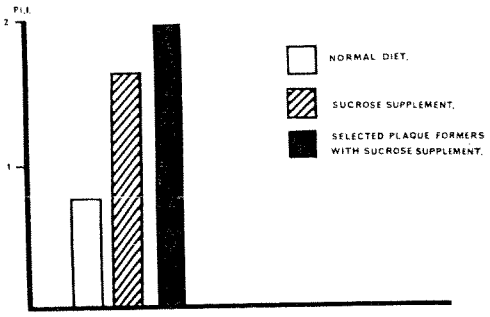

Fig. 2. The mean Plaque Index of groups of students after three days of no oral hygiene.

The clinical experimental series were performed in periods of 4 days, usually lasting from Monday to Friday. At the start of each period the participants were brought to Pl I = 0. During the test periods no oral hygiene was allowed, and in addition to the sucrose rinses 2 daily rinses with one of the antibacterial agents were instituted. The experiments were concluded with the recording of the Pl I. The disinfectants were randomly distributed on the test groups, all plaque estimations were performed by one person and the studies were carried out "double blind".

Bacteriological tests. The minimum lethal concentration tests of the different compounds were performed by two methods:
a) Surface contact tests (Fig. 3) were performed by flooding the surface of blood agar media in Petri dishes with saliva (diluted 1:10 in glucose broth). The excess fluid was drained off and the dishes dried for 30 minutes at 37°C. Then 0.1 ml spots of serial 2-fold dilutions of the disinfectants were applied. The highest dilution giving a total inhibition of bacterial growth was recorded after 24 hours of incubation at 37°C. Similar tests were performed with a mixed culture of oral streptococci and staphylococci as target bacteria.
b) Tests of the "phenol coefficient" type were carried out employing saliva as source of test organisms. To serial 2-fold dilutions of 1 ml of antibacterial agent in water was added 3 drops of saliva (diluted 1:10 in glucose broth). After 5 minutes of contact at room temperature a loopful of fluid from each culture was transferred to a Clark medium (glucose broth) and to a thioglycolate medium. The growth in these media was estimated after 72 hours of incubation at 37°C.

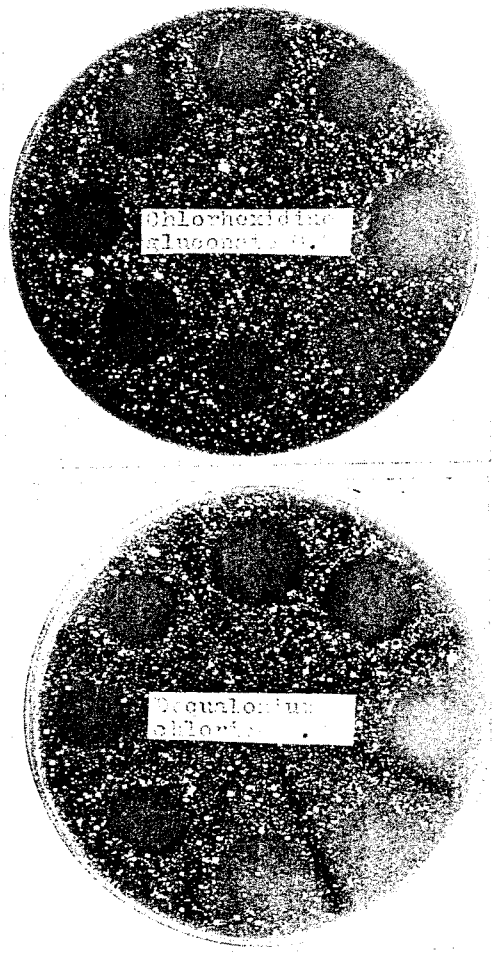

Fig. 3. Two-fold dilutions of disinfectants on layers of salivary bacteria. The stated concentration 3 o'clock, dilutions counterclockwise.

Results

Clinical tests. Chlorhexidine gluconate and chlorhexidine acetate almost completely inhibited plaque formation on the teeth under the experimental conditions of this study. Benzalkonium chloride also inhibited plaque formation, but not to the same degree as the chlorhexidine salts. All other compounds tested had a negligible effect on the amount of plaque formed during the test periods as compared with the controls. The Pl I values after the use of benzalkonium chloride in concentrations of 0.2 per cent and 0.1 per cent reflect a rather good effect of both concentrations, and a certain effect was also experienced with the use of chlorhexidine acetate 0.1 per cent. Proquanil hydrochloride showed a slight effect on the amount of plaque when 4 daily rinses were instituted (Table I). The distribution of the individual Pl I scores of the test persons and their variation within the test groups are shown in detail in Table II. The wide range of the individual Pl I scores of the 0.1 per cent groups of both chlorhexidine acetate and benzalkonium chloride are remarkable. Water mouthrinses were used as control and show the "normal" range of Pl I scores for the entire group.

Side effects. The test persons generally complained of the strong and bitter taste of the majority of the mouthrinses used, and some agents (the chlorhexidine salts) interfered with the taste sensation for some time after each rinse. In some cases the chlorhexidine salts led to slightly brownish discolourations of the teeth and the tongue as described by Löe and Schiött (1970 a, b). Similar effects were noticed after rinses with 0.2 per cent benzalkonium chloride. Four of the five persons in the 0.2 per cent benzalkonium chloride group developed ulcerative desquamative lesions on the oral mucosa. One test person (S.P.) contracted acute parotitis when rinsing with 0.2 per cent chlorhexidine gluconate.

Table I

Mean Plaque Index after rinsing with antimicrobial compounds

Antimicrobial compound	Concentration	n	$\bar{x}$	s. d.	Duration of experiments (days)
Control		16	1.75	0.10	4
Chlorhexidine gluconate	0.2 % (2,2 mM)	6	0.23	0.26	4
Chlorhexidine acetate	0.2 % (3,2 mM)	6	0.19	0.16	4
Chlorhexidine acetate	0.1 % (1,6 mM)	9	0.75	0.51	4
Jodosan®	0.1 %	5	1.95	0.05	6
Cetylpyridinium chloride	0.1 % (2,8 mM)	5	1.93	0.05	6
Dequalinium chloride	0.2 % (3,8 mM)	5	1.88	0.09	4
Benzalkonium chloride	0.2 % (5,6 mM)	5	0.40	0.19	4
Benzalkonium chloride	0.1 % (2,8 mM)	4	0.47	0.58	3
Aminacridine hydrochloride	0.2 % (8,0 mM)	5	1.43	0.26	3
Mepacrine hydrochloride	0.2 % (3,9 mM)	5	1.65	0.12	4
Proguanil hydrochloride	0.2 % (6,9 mM)	5	1.50	0.14	4
Proguanil hydrochloride	0.2 %	9	0.89*	0.34	4
Dibromopropamidine diisethionate	0.2 % (2,8 mM)	4	1.36	0.15	4
Hydrogen peroxide	3 %	4	1.15	0.43	3
Ethanol	50 % W/W	5	1.92	0.06	4

* 4 rinses a day

Table II

Distribution of mean Plaque Index values of the test persons after rinsing with antimicrobial compounds

Test persons	Chlorhexidine gluconate 0.2 %	Chlorhexidine acetate 0.2 %	Chlorhexidine acetate 0.1 %	Jodosan®	Cetylpyridinium chloride 0.1 %	Dequalinium chloride 0.2 %	Benzalkonium chloride 0.2 %	Benzalkonium chloride 0.1 %	Aminacridine hydrochloride 0.2 %	Mepacrine hydrochloride 0.2 %	Proguanil hydrochloride 0.2 %	Dibromopropamidine diisethionate 0.2 %	Hydrogen peroxide 3 %	Ethanol 50 % w/w	Control
O. M.	–	–	0.57	–	–	1.81	0.32	0.26	–	–	1.62	–	–	–	1.92
K. G.	–	–	0.32	–	1.93	–	–	–	–	1.73	–	–	0.60	1.94	1.90
G. E.	–	–	1.11	–	–	1.86	–	1.31	–	–	1.65	–	–	–	1.84
K. O.	–	0.32	0.46	2.00	–	–	–	0.34	–	–	–	1.31	–	–	1.83
Ø. J.	–	–	–	1.99	–	1.98	–	–	1.66	–	1.33	1.48	–	–	1.83
I. B.	–	–	0.21	–	1.96	–	0.13	0.21	–	–	–	–	–	1.97	1.81
T. Y.	0.08	–	0.17 –	1.92	–	–	–	–	–	–	–	–	–	–	1.78
T. N.	0.17 –	–	–	1.89	–	1.97	0.54	–	–	1.46	1.38	–	1.48	–	1.75
I. S.	0.75	–	–	–	–	–	–	–	–	1.76	–	–	1.50	–	1.74
L. E.	0.16 –	0.40	–	–	1.97	–	–	–	1.65	–	–	–	–	1.96	1.71
Ø. E.	–	–	1.39	–	1.93	–	0.61	–	–	1.71	–	–	–	1.86	1.69
E. B.	–	0.08	–	–	1.87	–	–	–	1.35	–	–	1.18	–	–	1.66
S. T.	–	0.02 –	1.14	–	–	1.78	–	–	–	–	1.54	–	–	–	1.66
S. P.	–	0.06	–	–	–	–	–	–	1.03	–	–	–	–	1.83	1.64
V. S.	0.04	–	–	–	–	–	0.40	–	–	1.60	–	–	1.03	–	1.62
K. K.	0.20 –	0.07	1.41	1.93	–	–	–	–	1.47	–	–	1.49	–	–	1.61
Mean	0.23	0.19	0.75	1.95	1.93	1.88	0.40	0.47	1.43	1.65	1.50	1.36	1.15	1.92	1.75

Bacteriological tests. Benzalkonium chloride and the dyes mepacrine- and aminacridine hydrochloride inhibited growth of salivary microorganisms on blood agar plates in a dilution 1:16, whereas the other compounds were effective in higher concentrations only (Table III). Streptococci and staphylococci were inhibited by high dilutions of dequalinium chloride, cetylpyridinium chloride, benzalkonium chloride and chlorhexidine gluconate and -acetate. The tests in liquid medium of the "phenol coefficient" type showed high activity by cetylpyridinium chloride and benzalkonium chloride and only moderate activity by the chlorhexidine salts (Table III). In the thioglycolate medium tests, benzalkonium chloride exbihited the highest activity. The chlorhexidine salts and the dequalinium chloride had a somewhat lower activity.

Discussion

The pilot study prior to the experiments confirmed the observation by Schroeder (1969) of certain intra-individual variations in the plaque formation rate. The test persons in this study consisted of selected individuals rated as heavy plaque formers. Sucrose rinses were introduced for two reasons: 1) To accelerate plaque formation (Carlsson and Egelberg 1965b, Carlsson and Sundström 1968) and 2) to induce a kind of plaque closely related to caries (Bowen 1970). Several other reports indicate that

Table III

The effect of antimicrobial agents used as mouthrinses on salivary bacteria *in vitro* (minimum lethal consentration), and their corresponding mean Plaque Index values

Antimicrobial agent	Media / source of bacteria				
	Blood agar / saliva*	Blood agar / oral strepto- and staphylococci	Clark / saliva*	Thioglycolate / saliva*	Mean Pl. I.**
Chlorhexidine gluconate 0.2 %	1:2	1:64	1:32	1:8	0.23
Chlorhexidine acetate 0.2 %	1:8	1:64	1:32	1:8	0.19
Jodosan®	1:4	1:8	1:16	1:2	1.95
Cetylpyridinium chloride 0.1 %	1:4	1:64	1:256	1:4	1.93
Dequalinium chloride 0.2 %	1:2	1:128	1:4	1:8	1.88
Benzalkonium chloride 0.1 %	1:16	1:64	1:128	1:32	0.47
Aminacridine hydrochloride 0.2 %	1:16	n. e.	n. e.	n. e.	1.43
Mepacrine hydrochloride 0.2 %	1:16	n. e.	n. e.	n. e.	1.65
Proguanil hydrochloride 0.2 %	1:2	n. e.	1:1	1:1	1.50
Dibromopropamidine diisethionate 0.2 %	1:1	1:32	n. e.	n. e.	1.36
Hydrogen peroxide 3 %	n. e.	n. e.	1:2	n. e	1.15
Ethanol 50 % w/w	n. e.	n. e.	n. e.	n. e.	1.92
Control					1.75

* Diluted 1:10
** Control Pl. I. = 1.75
n. e. = not effective.

sucrose induced plaque, initiates rapid caries and periodontitis (Shaw and Griffiths 1961, Carlsson and Egelberg 1965a).

The results of the clinical experiments confirmed previous reports that the chlorhexidine salts (acetate and -gluconate) have a strong inhibiting effect on plaque formation (Schroeder 1969 and Löe and Rindom Schiött 1969, 1970a, b). However, a *total* inhibition was not experienced in the present study employing heavy plaque formers and sucrose rinses. Benzalkonium chloride also showed a rather good inhibiting effect on plaque formation. Both the chlorhexidine salts and the benzalkonium chloride had a bactericidal effect in high dilutions on salivary bacteria in our *in vitro* tests systems. However, several compounds with equally good or better *in vitro* activity did not inhibit plaque formation *in vivo* (dequalinium chloride, cetylpyridinium chloride, mepacrine hydrochloride, aminacridine hydrochloride, Jodosan®). In a related *in vivo* system the same iodophore was tested by Strålfors (1961) with no effect, and dequalinium chloride has also proved ineffective in previous experiments (Schroeder 1969). The plaque-inhibiting effect by cetylpyridinium chloride reported by the same author was not confirmed in our experiments. This discrepancy may be due to the use of selected plaque formers and the supplement of sucrose in the present study.

The bacteriological *in vitro* tests will at their best only serve as very simplified models of the *in vivo* conditions in the mouth. The data obtained in 4 different systems in the present study are relevant as far as the

types of bacteria are concerned, and the presence of salivary proteins in most of the systems is probably an important contribution to the imitation of the *in vivo* conditions (most agents killed salivary bacteria to a higher titre when salivary proteins were not present). Assuming that these *in vitro* tests to a certain extent have a relation to the antibacterial effects of the tested compounds in the mouth, the results of the present experiments indicate that there is no direct correlation between the antibacterial activity against salivary bacteria and the inhibiting effect on plaque formation. Factors other than the general antibacterial activity are presumably important. Schroeder (1969) held a selective effect by some disinfectants on the plaque-forming bacteria to be important, whereas others have suggested that effects like affinity for hydroxyapatite or salivary mucins might be a basis for plaque inhibition *in vivo* (Rölla, Löe and Rindom Schiøtt 1970). Proguanil and dibromopropamidine were included in our experiments because of their chemical relationship with chlorhexidine. No effects of these compounds comparable with that of chlorhexidine coul be demonstrated.

Schroeder (1969) observed a sort of "all or none" response in his group using 0.1 per cent chlorhexidine acetate. This observation was confirmed in the present study (Table II), whereas an increase in the concentration to 0.2 per cent gave inhibition in all persons tested. Chlorhexidine forms salts with anions and polyanions (Rölla *et al.* 1970), some of which have a low solubility. High levels of phosphate, chloride or acidic proteins (mucins) in the saliva of some individuals may act as "neutralizing agents" impeding the effect of low concentrations of chlorhexidine.

The painful lesions observed after the use of benzalkonium chloride in the mouthrinses seem to exclude the use of this agent in preventive dentistry.

The test person (S.P.) who contracted acute parotitis during the test of chlorhexidine gluconate (0.2 per cent) had not experienced virus parotitis previously, and the symptoms indicated that he was suffering from this disease. A later test of chlorhexidine acetate on the same person did not cause any complications. Presence of detergents in mouthwashes has otherwise in some cases caused precipitation of protein and stenosis in salivary ducts with subsequent painful gland tumor.

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